September 24, 1965

Dear Paul:

The contract just executed for literature search on vision and perception stipulates that we shall report at once any work that appears to be of special significance. I am taking advantage of this clause of the contract to report briefly some recent important findings about the mechanism of wavelength discrimination, which I learned of while doing pre-proposal background study, and to submit for your consideration some tentative views on methods of collecting, reporting, and evaluating data under the program.

In the early nineteenth century it was theorized that the retina of the vertebrate eye probably contains three different light-sensitive substances, each with a peak sensitivity in a different region of the spectrum, and that the information from the excitation of each substance is separately transmitted to the brain and combined there to reproduce the colors of the outside world. This theory was supported by rather strong inferential evidence, notably color-matching experiments which showed that people with normal vision can match any given spectral color by combining three primary colors in different parts of the spectrum. However, other psychological studies seemed to support rival theories, and the matter remained doubtful until just last year.

The tri-receptor hypothesis of wavelength discrimination has been confirmed by physiological experiments on goldfish, carried out at Johns Hopkins University in 1963 and reported in 1964. The article by MacNichol which describes this work is brief enough for office reproduction, so I am enclosing a copy which I think you should keep as a primary reference in your library. (In looking over this article, and indeed throughout the program, you will notice that physiological research on vision harps strangely upon goldfish. The reason for this is that only monkeys, some of the bony fishes, and man possess a fovea, the tiny central area of the retina in which cone receptors are densely packed and which mediates high-acuity vision.)

The author's rather sobersided style may obscure the fact that his work is as basically significant in vision research as, say, the wave theory of light. In the course of four or five years, this work will find its way into textbooks and other general references, and its scientific importance will be suitably emphasized. For us, however, the question immediately arises: what is the bearing of such information, however scientifically significant, on the subjective experience of the photo interpreter or other worker doing a visual task? I am afraid there is no categorical answer to this question.

In the work statement of the proposal I listed among the subjects to be investigated "mechanisms of wavelength discrimination and subjective phenomena of color perception." For the evaluation of data on these subjects, it is important to realize that wavelength discrimination in the retina does <u>not</u> result directly in color perception in the mind, and that the relations between the two events are indirect, tenuous, and still largely unexplained. Some facts about physiological wavelength discrimination lend themselves to direct interpretation in terms of subjective experience. Other facts are part of a complex nervous organization for transmitting information to the brain, and only confusion can ensue from attempts to force psychological interpretations on them. For example, from the efficiency curve of wavelength discrimination (which peaks in the green) it does not follow that the photo interpreter "sees" green better than he "sees" red, his perception being conditioned by the interactions of adaptation, light intensity and quantum phenomena, spatial and emotional properties of color, and constancy effects. Subjective color perception is fraught with such complications, which make it a highly ambiguous, though interesting, subject for study. We could easily spend on color perception the entire time authorized for the program -- in fact it would be tempting to do so -- but in terms of the uses of color in working situations, we might not be much wiser than before.

I suggest, therefore, that the information on wavelength discrimination and color perception which we accumulate in the present program should be regarded, not as material for direct application, but rather as background knowledge, which needs to be better integrated before it can be successfully exploited. Unless I receive different instructions from you, I intend to collect and report the basic facts about color perception, bringing in physiological information where it seems important in relation to subjective experience. When you review these basic facts you can tell me which of them seem worthy of more intensive study. For example, I do not intend to make a close study of defects in color perception, unless expressly instructed to do so, on the assumption that color defects are not particularly relevant to your working problems. I do intend to study and report the experiments of Land in two-color projection, and any commentaries and criticisms of these experiments which I find in open literature, on the assumption that you are actively interested in the possible uses of color projection and the creation of artificial color worlds. However, I do not suggest that you should put this material to practical use -- even in an experimental sense -- without a firm grasp of the physical and psychological principles that are embodied in Land's results.

The reports which I plan to submit will not be chronological descriptions of work performed, but rather topical essays, each dealing with a particular aspect of vision or perception. I should like first to submit a brief report on visual acuity and the nature of visual thresholds; I believe that fundamental misunderstanding of these concepts is at the root of many theoretical and practical problems of photo interpretation. This first report will give you a chance to review my general method of approach and supply any redirection that you may feel is necessary. The report on color perception will be much more complex and difficult, and will have to await accumulation and study of material as well as refinement of objectives.

I look forward to discussing the above ideas with you when you come to visit us, and to showing you some of my preliminary material.

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RETINAL MECHANISMS OF COLOR VISION[1]

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Abstract—Over a century and a half ago YOUNG (1802) theorized that color discrimination is mediated by three photoreceptor substances, each maximally sensitive to a different region of the spectrum, and that the activity of each of these substances is somehow communicated in a unique fashion to the brain. In the last few years the techniques of electrophysiology and retinal spectrophotometry have made possible experiments which justify Young's conclusions. In particular, the technique of single-cell microspectrophotometry has revealed that in the goldfish, an animal known from behavioral experiments to have a well-developed ability to discriminate colors, there are three groups of retinal cones, each containing a single pigment maximally sensitive in a particular region of the spectrum. Although electrophysiology has not yet yielded much definite information in regard to photopigments, since it has not been possible to record from single cones, it has provided much information on the function of the neural layers of the retina. The most distal responses recorded with microelectrodes, the *S*-potentials, are slow, graded potentials in response to illumination. One kind, the *C*-response, has opposite polarity in different regions of the spectrum. The retinal ganglion cells which initiate impulses in the optic nerve fibers are acted upon by paired excitatory and inhibitory influences from groups of receptors maximally sensitive in different regions of the spectrum. Thus, whereas the receptors have been demonstrated to behave in a manner consistent with the trichromatic theory, the neural responses are encoded in a fashion consistent with Hering's opponent color hypothesis.

INTRODUCTION

IN MODERN Physics, when new theoretical predictions are made, direct and satisfying results that give unequivocal and clear-cut answers are frequently obtained in a year or two; often through Herculean efforts requiring mile-long vacuum tunnels, thousands of tons of magnet iron and shielding, and expenditures of gigawatt hours of electrical energy and many millions of dollars. Biological science is not usually as fortunate in obtaining such prompt results: the theoretical question which forms the basis of this discussion was posed by Thomas Young in 1802. The direct and unequivocal answers have just started coming in, over a century and a half later. As in Physics, the results have been made possible by advanced physical instrumentation which has been available for only a very short time. This equipment, though sophisticated, is fortunately not of the monumental size and complexity required by the physicists.

Young stated that it was unlikely that there was a special kind of receptor (vibrating particle) for each small wavelength region in the spectrum and that the then known facts of color-mixing suggested that there was only a small number of receptor types involved, probably only three. He further hypothesized that these receptors each communicate their activity to the brain in some unique manner. Psychophysical experiments during the following century and a half have amply demonstrated the trichromacy of vision; for example, it has been convincingly shown that the entire span of spectral colors can be exactly matched by mixtures of lights of any three primary wavelength bands, provided

[1] This paper is based on material presented orally to the 17th International Congress of Psychology, Washington, D.C., 21 August 1963.

only that they are chosen from appropriate regions of the spectrum. There are of course some complications: to match some regions of the spectrum the energy in one of the primary wavelength bands must have a negative value; that is, it must be added to the light being matched rather than to the matching light. There is also the problem of the uniqueness of the sensation of yellow, and that while there are bluish greens there is no such thing as a reddish green or a yellowish blue. Such considerations led to the Hering opponent color theory and its derivatives. In addition, it has been found that a wide variety of color sensations could be elicited with less than three primary colors, particularly when the test object was a complicated photographic scene. In fact, the startling demonstrations by LAND (1959) show that the colors of most objects in a photograph can be correctly named when subjects are presented with an image that consists only of mixtures of red and white light in various proportions. In spite of these complications the basic facts are indisputable: all colors can be matched by mixtures of three primary colors in various proportions. Abnormalities in color vision can be accounted for by the lack of discriminability of one or more of the primary colors. The questions that psychophysical experiments have not been able to answer in a direct and unequivocal manner are how the three primary wavelength regions are discriminated and encoded in the retina for transmission along the optic nerve; and how this information subsequently is decoded in the brain.

Thanks to modern physical apparatus, these questions are beginning to be answered in a simple and direct way. Young hypothesized that the retina must be affected in three different ways by the three primary colors. This could be brought about in a number of ways; possibly there are three different kinds of receptors. Each would contain a photosensitive substance with maximum sensitivity in a different region of the spectrum, or would contain a single substance on which only a selected region of the spectrum is allowed to fall, due to the interposition of some sort of an optical filter. Alternatively, there might be a single kind of receptor that contains three different kinds of pigment, or a single pigment that is somehow affected differently by lights from three wavelength regions. This single kind of receptor would in some unknown way produce nervous effects that were different for the different colored lights it received. The filters could be pigments similar to those found in the colored oil droplets of the cone ellipsoids of birds, or they could be physical filters such as multilayer interference filters or filters based in the "waveguide" propagation modes observed by ENOCH (1963) in receptor outer segments. For example BIERNSON (1963) has proposed a single receptor theory in which a physical filter is scanned through the visible spectrum at about 20 c/s. The color information is recovered by a neuronal phase detection circuit. The theory is entirely consistent with the facts of color-mixing and of color defect. It was so constructed. However, the three-receptor hypothesis is at least equally plausible. Obviously, some direct, objective experiment is needed to determine whether or not there are in fact three or even more kinds of cone, or only one.

Cone pigments are difficult to extract and have not been separated chemically from one another. This fact favors hypotheses involving a single receptor pigment. However, the pigments would presumably differ only slightly in the structure of the protein part of the molecule and, furthermore, the pigments are not directly soluble. Thus, separation by the usual biochemical methods such as precipitation, chromatography or electrophoresis has not yet been accomplished. Analysis of pigment mixtures in solution by the partial bleaching technique has not yielded a clear indication of different kinds of cone pigments in higher vertebrates, although it was successful in distinguishing the cone pigment iodopsin from the rod pigment rhodopsin, in a mixture of the two (WALD *et al.*, 1955). Thus, new methods were required for distinguishing them *in situ*. The first successful method was that of CAMPBELL and RUSHTON (1955), who constructed a device for measuring the intensity of light reflected from the living human fundus after having passed twice through the retina. RUSHTON (1958) reported finding two photolabile pigments in the rod-free area of the fovea. One of these was maximally photosensitive in the yellow (chlorolabe) and the other in the red (erythrolabe). He measured the kinetics of bleaching and regeneration, finding that they agreed well with those of iodopsin in solution. These pigments also agreed well (provided certain assumptions were made) with the receptor sensitivity functions determined by STILES (1959) who used psychophysical method involving light-adaptation at various wavelengths (RUSHTON, personal communication, 1963; MARRIOTT, 1962). Unfortunately reflection densitometry on areas of the retina containing large numbers of receptors does not tell us whether the pigments occur in different receptors or are mixed in a single type of receptor, or whether there is a single pigment with several kinds of filters in front of it. BRINDLEY and RUSHTON (1959) ruled out the filter hypothesis by showing that accurate color matches could be made between light entering normally through the pupil and light entering from behind through the sclera. Unless the filter completely surrounded the outer segments of the receptors such a match would be impossible.

SINGLE RECEPTOR SPECTROPHOTOMETRY

Clearly, the simplest way conceptually to distinguish between the single receptor and multi-receptor hypotheses is to measure the absorption or action spectra of photopigments *in situ* in single receptors. Absorption measurements are difficult since cone outer segments have a volume of only a few μ^3 and their absorption turns out to be less than, at most, 10 per cent of the incident light. Some preliminary work on carp cones was reported by HANAOKA and FUJIMOTO (1957) who indicated that there are at least five different kinds of cones having absorption maxima at about 495, 530, 570, 630 and 680 mμ. For some reason these authors have not published any more work in this field and what they did publish conflicts with our own more recent work. BROWN (1961) and LIEBMAN (1962) have subsequently published microspectrophotometric measurements on the outer segment of frog rods. However, their instruments apparently do not have sufficient sensitivity to detect photopigments in the much smaller outer segments of cones. WOLKEN (1962, 1963) was able to obtain a composite measurement of the spectral response of frog cones. He used a separate cone to measure absorption at each wavelength since the light he needed for his measurements was so strong that it bleached the pigments very rapidly. This technique, because it used a population of receptors to obtain a single curve, cannot, of course, distinguish between different kinds of cones in the same retina.

An instrument sensitive enough to measure spectral absorption curves of small cones without irreparable distortion due to bleaching has been constructed by MARKS and MACNICHOL (1962, 1963) and MARKS (1963). This work will be described more fully in a forthcoming publication.

Figure 1 shows a cone of the stage of our microspectrophotometer. The arrows indicate two spots of light, the measuring beam and a reference beam.

In the absence of any photopigment the energy received by the light-measuring system in our instrument from the two illuminated spots is not equal throughout the spectrum. This is due to the effects of stable pigments and wavelength dependent scattering by particulate matter in the receptors; chromatic aberration and diffraction effects in the optical system.

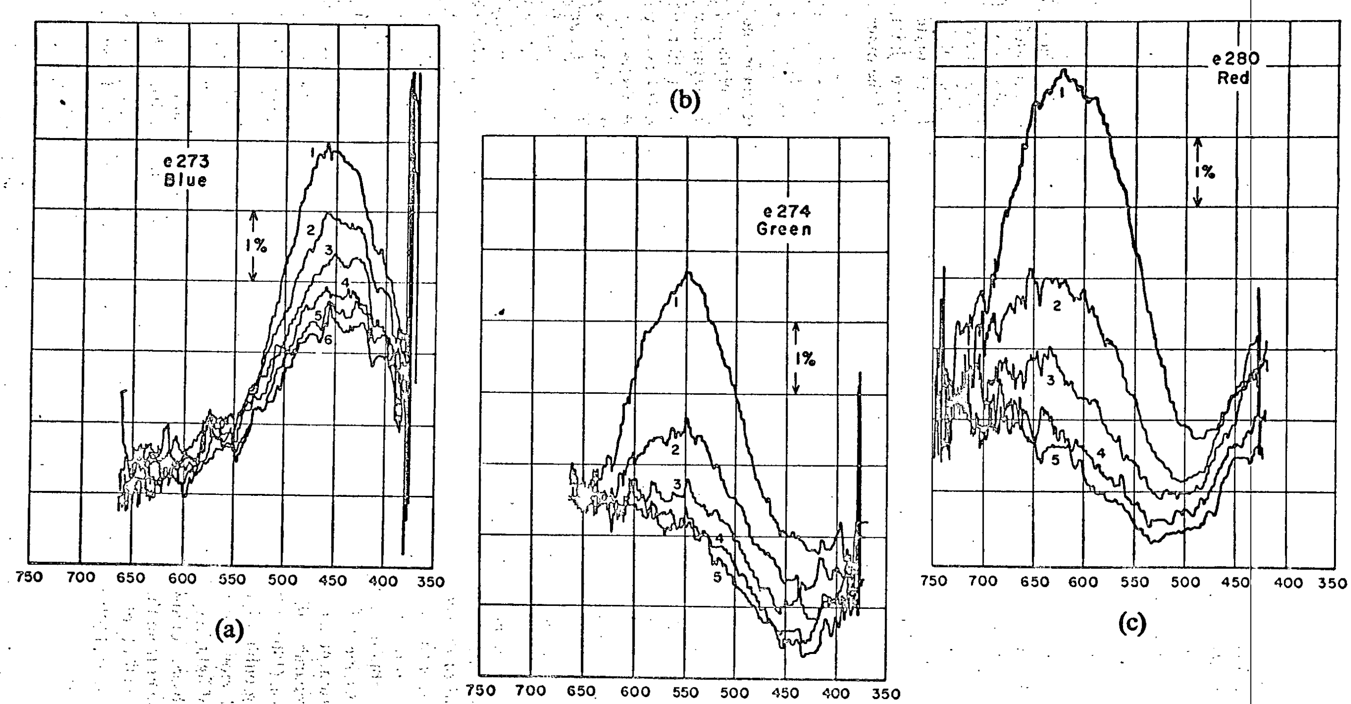

FIG. 2. Absorption spectra of 3 cone outer segments having maximum absorption in 3 different regions of the spectrum. The vertical scale divisions indicate percentage absorption. The spectrum was scanned from long to short wavelengths in a and b, from short to long wave- lengths in c. The differences between the first and last curves in the records are maximal at 455, 545 and 610 mμ. Correction for progressive bleaching moves the maxima to about 450, 530 and 625 mμ.

To minimize these effects bleaching difference spectra are obtained. These are classically determined by first obtaining an absorption spectrum, then bleaching the photosensitive pigment with white light, obtaining a second absorption spectrum, and subtracting the absorptions at corresponding wavelengths. Thus, provided colored photoproducts are not produced (often they are and must be corrected for) the difference spectrum should give the true absorption spectrum of the bleachable pigment. In our measurements sufficient light is used to bleach about half the photopigment during a single scan through the spectrum. Subtracting absorption at corresponding wavelengths between a pair of absorption spectra made on a single receptor gives an uncorrected bleaching difference spectrum for the photopigment. The difference spectrum must then be corrected for the loss of pigment molecules during a single measurement.

Figure 2 shows the raw data obtained for three different cones from goldfish retinae. It is evident that they indicate maximum absorption by different receptors in three distinct regions of the spectrum. The maxima of these curves are displaced from their true wavelength because there is progressively less pigment available as the measurement continues. Each absorption value can be corrected by dividing it by an estimate of the fraction of pigment remaining at that time. Marks has worked out a correction procedure which consists of first obtaining a difference spectrum and estimating the amount of pigment remaining as each wavelength was scanned. The curves are also smoothed by averaging a number of adjacent data points and plotting the average as the center point. A computer

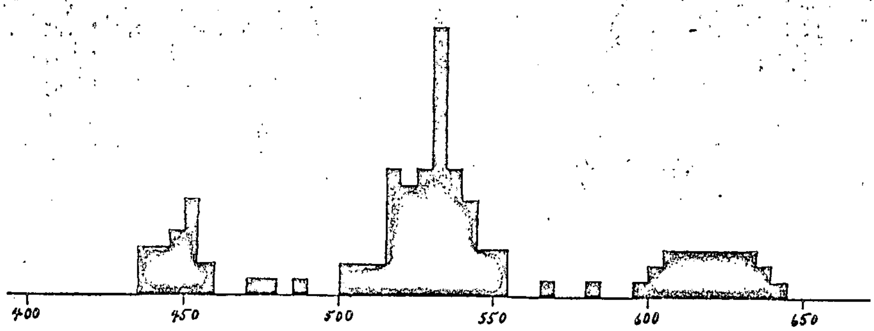

FIG. 3. Histograms from 113 experiments on single cones such as those shown in Fig. 2, showing the number of occurrences of cones having maxima in their corrected difference spectra at a given wavelength plotted as a function of wavelength. The entries at 565 and 580 mμ are the peak wavelengths of composite spectra of overlapping twin cones. Those at 470, 475 and 485 mμ are not explained. Otherwise the receptors can be seen to fall into three distinct groupings. (From MARKS, 1963.)

program for accomplishing this smoothing of curves (and estimating the number of photons absorbed by the visual pigment and its photoproducts) was worked out by Marks in collaboration with Dr. W. E. Love. The spectrophotometric data is automatically digitized and punched on paper tape as it is being recorded. The computer then makes the necessary calculations and plots the results by means of an electric typewriter. Figure 3 is a histogram of the corrected absorption maxima from 113 experiments, each on an individual cone. They were not selected in any way. It is evident that the population falls into three groups. The maxima of the three distributions are at 455, 530 and 625 mμ.

The two measurements that gave spectral maxima at 565 and 580 mμ are composite spectra of twin cones and thus do not represent a single pigment.

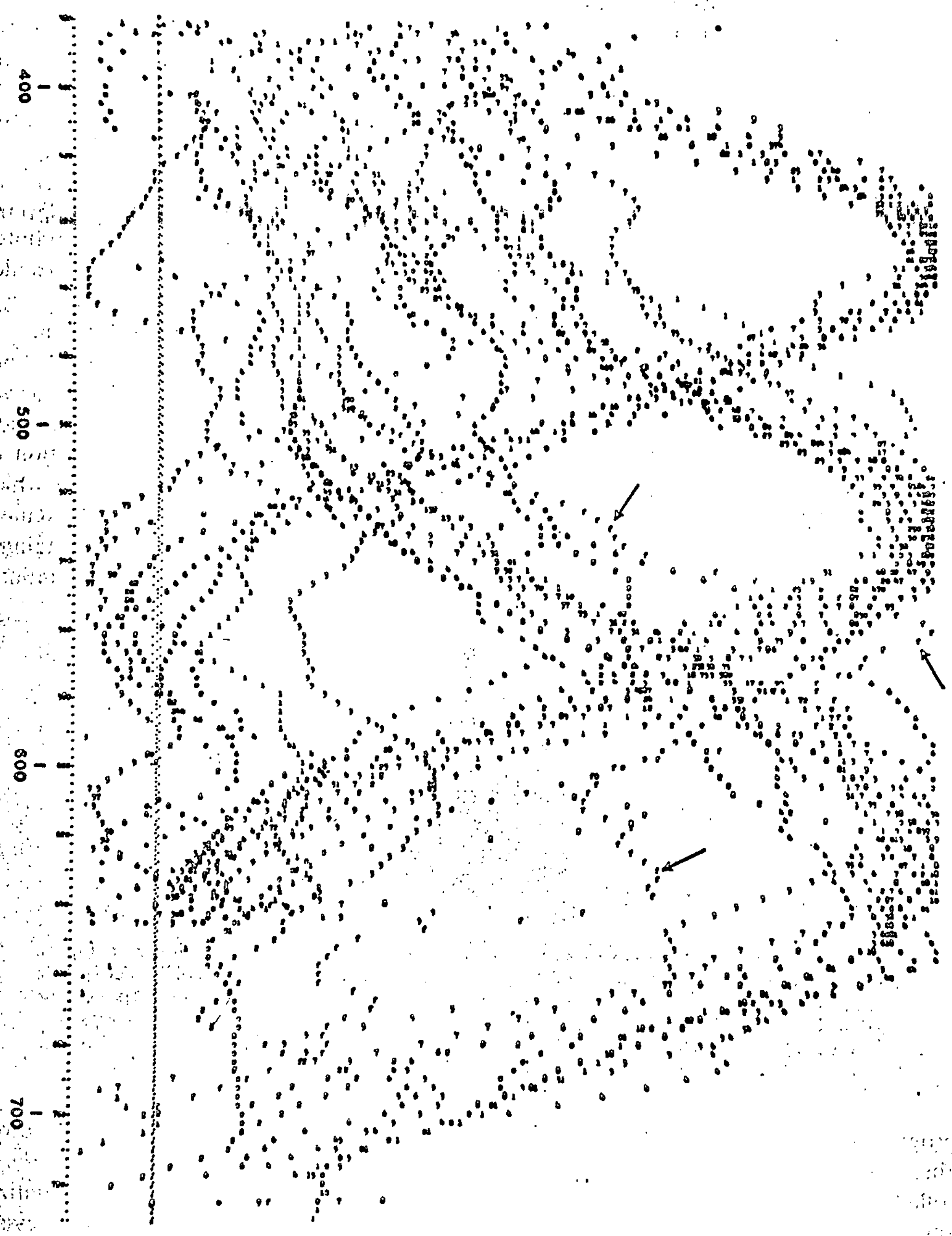

FIG. 4. Corrected transmission difference spectra of 28 experiments selected only for signal-to-noise ratio and normalized to the same height. Curve f (arrows); the composite spectrum of a pair of touching twin cones shows the greatest departure from the center of a group. The horizontal dotted line represents zero difference in bleaching. A number of the spectra that peak in the blue become negative at the center of the spectrum and then go through submaxima in the red region. (From Marks, 1963.)

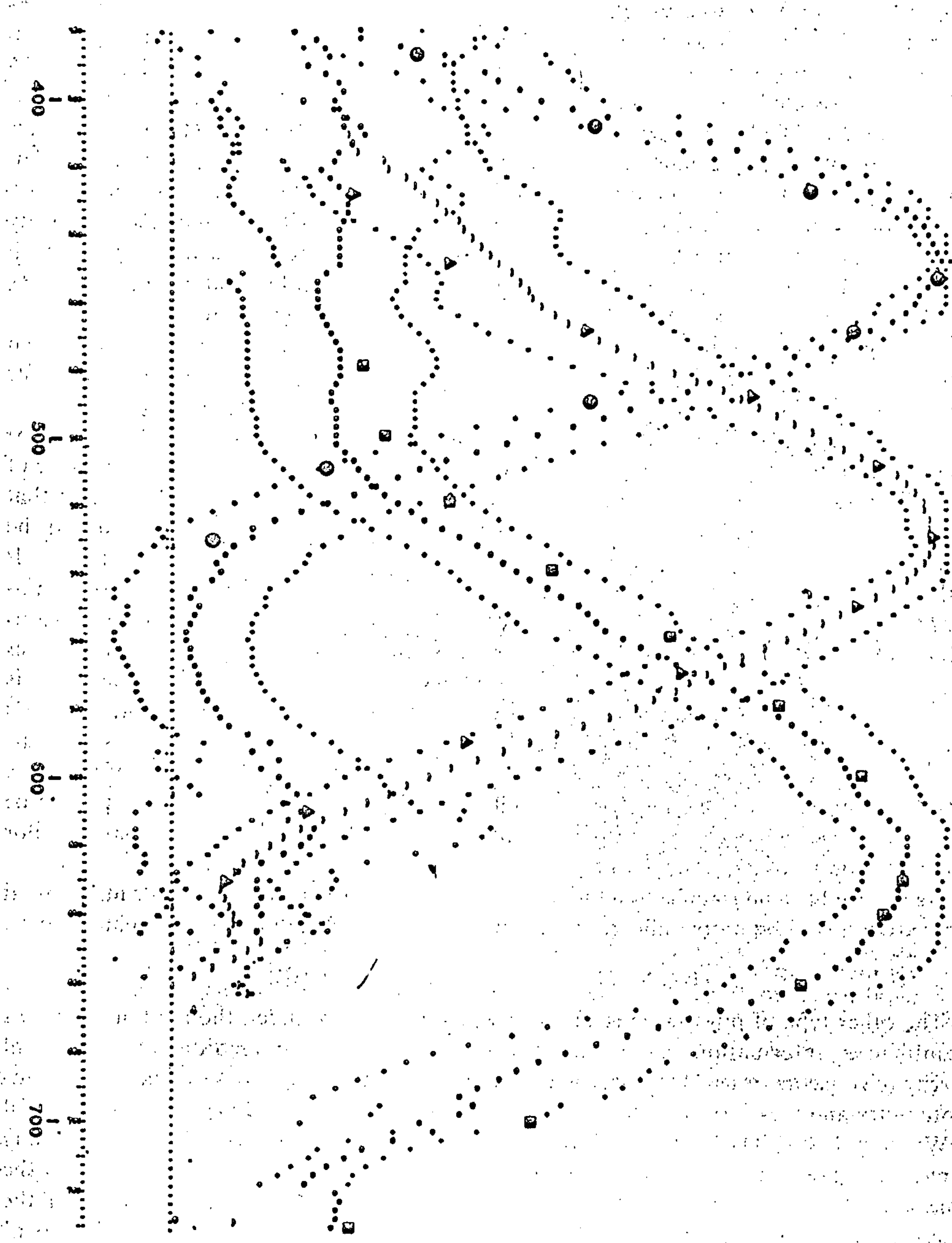

FIG. 5. Averages of the three groups of difference spectra shown in Fig. 4 excluding curve f. The bracketing curves show the standard deviation of the data. The large spots (filled circles, triangles and squares) are for hypothetical pigments derived from the DARTNALL (1953) nomogram plotted to have maxima at 455, 530 and 625 mμ.

Some idea of scatter in the data can be obtained from Fig. 4, which is a plot of the computed spectra from twenty-eight experiments which gave the greatest maximum absorption but which were not selected in any other way. It is evident that they fall into three close groups, except for (f) which is the composite spectrum of a pair of twin cones. Figure 5 shows the average of the curves in each of the three groups and the standard deviations of the data points. The large spots are points derived from the DARTNALL (1953) nomogram for three hypothetical pigments having the maxima of the three averaged curves, 455, 530 and 625 mμ. The Dartnall nomogram is based on rhodopsin. Porphyropsin, iodopsin and other visual pigments that have been extracted are consistent with it. The relative density function of these pigments is invariant if plotted on a wave number rather than a wavelength scale. It can be seen that the green absorbing pigment is a typical Dartnall pigment, whereas the blue pigment has a somewhat broader and the red a narrower absorption.

An explanation for the narrower absorption of the red-sensitive pigment has been suggested by Lewis and some of its implications confirmed by Brindley and by Stiles (for discussion, see BRINDLEY, 1960).

Although many details need to be explained, a direct and unequivocal conclusion can be drawn from our experiments: for at least one species known behaviorally to be capable of color discrimination (MCCLEARY and BERNSTEIN, 1959). Thomas Young's prediction that there are three kinds of receptors has been verified. In addition, Marks has found that the pigment density in cones is about 10^6 molecules/μ^3, which is nearly the same as in rods. It is also present throughout the outer segments and the chromophores are aligned perpendicular to the axis of the receptors as in rods (SCHMIDT, 1938; LIEBMAN, 1962). Thus previous failures to extract large quantities of cone pigments can no longer be explained on the basis suggested by RUSHTON (1962), that each cone contains a minute granule of pigment that is used efficiently because of a focusing effect of the ellipsoid and outer segment. Marks's measurements were made with the receptors on their sides, light entering transversely to the axis. Furthermore, it was possible to make measurements on several portions of the same outer segment with identical results. Thus filtering and focusing, while they may play some part, as for example in accounting for the Stiles–Crawford effect, are not essential for color discrimination in the goldfish.

As yet, we have no significant data on human or other primate receptors. Primate foveal cones are smaller and more difficult to measure, but we have high hopes of ultimate success.

THE MICROELECTRODE TECHNIQUE

The other type of physical measurement which is yielding direct, though not always as unambiguous information, is the microelectrode technique for recording the electrical activity of single neurons. This method has been in use much longer than single cell spectrophotometry and was first described independently by GRANIT and SVAETICHIN (1939) and by WILSKA (1940). These workers developed their microelectrodes specifically for recording from retinal ganglion cells only a short time after HARTLINE (1938) had accomplished the same result by the more difficult dissected fiber technique. Since its development the microelectrode has been used successfully to trace the nerve message through many parts of the visual pathway as well as in other parts of the nervous system. In this paper only its contribution to our knowledge of the mechanism of color discrimination will be discussed.

Electrical responses have been demonstrated in some invertebrate photoreceptor cells. Especially convincing are the experiments of HAGINS *et al.* (1962) who clearly demonstrated local potential generated only in the illuminated region of squid photoreceptor cells.

The results with vertebrate photoreceptors are not nearly so convincing. It used to be assumed that a major portion of the electroretinogram was generated in the receptor cells. Recent work with microelectrodes (TOMITA, 1963) has indicated that most, if not all, the ERG is generated in other structures. Even Brown, who for a long time has been perhaps the strongest proponent of the idea that the *a*-wave or PIII process is a receptor potential, has been forced by his own experimental evidence to conclude that the source of this potential is no more distal than the receptor synaptic endings (BROWN and WATANABE, 1962). All that can be said at the present time about the mechanism of excitation and conduction in vertebrate photoreceptors is that very little is known about it and that it presents a real challenge to investigators. A few leads have been suggested by WALD *et al.* (1963), by McCONNELL and SCARPELLI (1963) and by JAHN (1963).

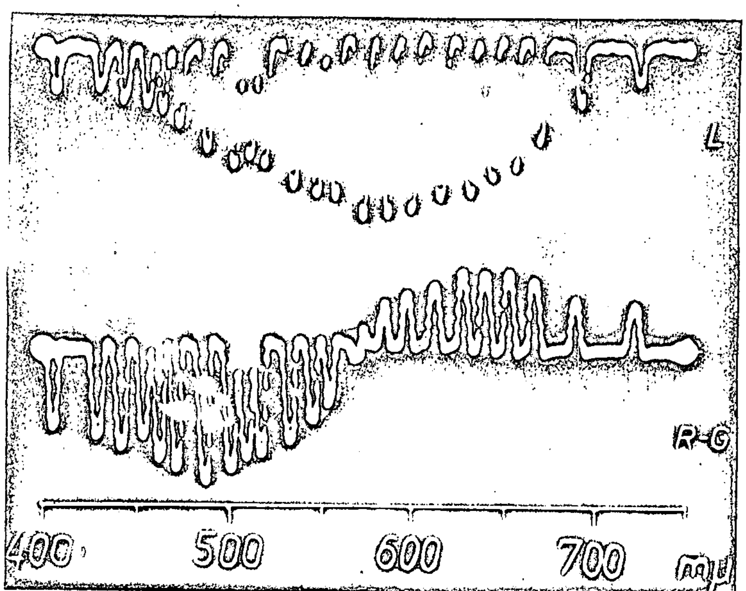

FIG. 6. Typical *S*-potentials in response to flashes of light of constant energy and progressively increasing wavelength plotted as a function of wavelength. Upper trace: Luminosity (L) response showing negative (downward) deflection only. Lower trace: Chromatic (C) response with both negative and positive components. (From SVAETICHIN and MACNICHOL, 1958.)

The most peripheral signs of color-related electrical activity that have thus far been described in the vertebrates are the *S*-potentials which were found by SVAETICHIN (1956) in fish retinae. Initially these were thought to be receptor potentials, a conclusion questioned by TOMITA (1957). Further work (MACNICHOL and SVAETICHIN, 1958) showed clearly that the potentials arise more proximally. The *S*-potentials are of two types as shown in Fig. 6. The most distal, known as the luminosity or *L*-response, is a negative hyperpolarization of the structure responsible, and has a broad spectral maximum. Histological electrode marking techniques show that it arises from giant horizontal glial cells which are abundantly present in the fish retina. Similar potentials have been obtained in the cat (GRÜSSER, 1957) and the frog (TOMITA *et al.*, 1961), which presumably have homologous, although much smaller, cells in their retinae. The color-related *S*-potential is called the *C*- or chromatic response. This potential is in the hyperpolarizing (negative) direction in part of the spectrum and depolarizing (positive) in other parts of the spectrum. Stimulation at a particular wavelength or with white light gives no sustained response, but only brief transients at the beginning and end of the stimulus period. The *C*-responses appear to be signs of a definite color discrimination mechanism which is reminiscent of the Hering opponent color hypothesis. In at least one fish, the mullet (*Mugil*) there are two kinds of *C*-response, which are shown in Fig. 7: a red–green opponent pair and a blue–yellow pair. There are also more

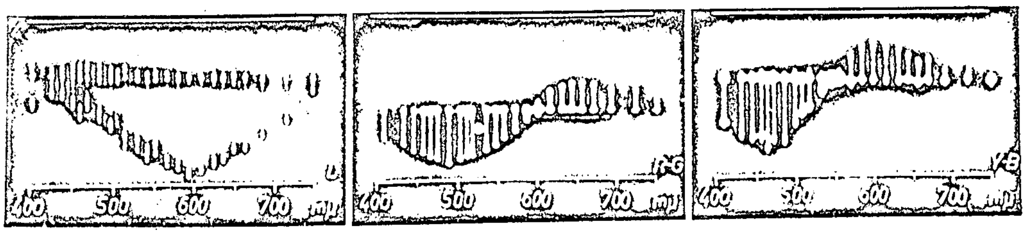

FIG. 7. *S*-potentials from the Mullet (*Mugil*) showing luminosity response and two kinds of chromatic responses. (From MACNICHOL and SVAETICHIN, 1958.)

complicated triphasic responses found in some species of fish (SVAETICHIN *et al.*, 1961; TOMITA, 1963; MOTOKAWA *et al.*, 1961). The *C*-responses were found to arise from more proximal structures than those giving rise to the *L*-responses and to occur in much more highly localized regions (MACNICHOL and SVAETICHIN, 1958). SVAETICHIN *et al.* (1961) have shown by histological electrode marking methods that they arise in Müller fibers which are glial elements surrounding the bipolar cells.

It is clear that the *S*-potentials are quite different in nature from the kind of electrical activity usually ascribed to nerve cells. They are steady potentials which are sustained during strong illumination and which adapt but little to a prolonged stimulus. SVAETICHIN *et al.* (1961) have stated the belief that they are signs of metabolic changes in glial cells induced by the activity of adjacent neurons. The altered metabolism of the glial cells would, in turn, alter the activity of the neurons. These observations give promise of opening up a whole new field in Neurophysiology: the study of neuron–glial interaction. Traditionally, the glia have been considered to play only a passive rôle as supporting structures in the nervous system. However, the electron microscopists have shown that there is very little extracellular space in the retina or in any part of the central nervous system. Ions and metabolites must pass through glial cells to get in and out of neurons. These cells when stimulated by neuronal activity are therefore in a position to exert regulatory and control functions on the activities of the nerve cells. The exquisitely sensitive microchemical studies by HYDÉN and PIGON (1960) have indicated that there are changes in DNA and other constituents of the glia which correlate with nervous activity. At present we can only say that we know very little about their significance, and that this is a very challenging area for further experimentation.

If the *S*-potentials have any significance in color vision they should bear some relation to the pattern of discharge in the fibers of the optic nerve which appears to be the only way in which the information collected by the retina is communicated to the brain. The first indication that there was such a relation was provided by DE VALOIS *et al.* (1958), who showed that many of the neurons in the lateral geniculate body of the monkey responded only at the beginning of a prolonged stimulus ("on"-response) at one extremity of the spectrum and only after the end of the stimulus ("off"-response) at the other extremity of the spectrum. At intermediate wavelengths there was an "on–off" response with the proportion of "on"- to "off"-impulses changing with wavelength. GRANIT (1948) had previously described this change in "on–off ratio" in the retinal ganglion cells of the cat, but presumably due to his preoccupation with the Dominator-Modulator theory did not assign it an important rôle as a mechanism of color discrimination. From the known facts of electrical excitation of neurons one would expect that at wavelengths where the *C*-responses are depolarizing there would be an "on"-response in the retinal ganglion cells. Where they are hyperpolarizing one would expect inhibition during illumination followed by an "off"-response. Unfortunately, no one has yet recorded *C*-responses from the monkey retina. However, they are obtainable from the goldfish and other Cyprinids. Accordingly, WAGNER *et al.* (1960, 1963), MACNICHOL *et al.* (1961) and WOLBARSHT *et al.* (1961) undertook a study of the effects of wavelength on the responses of retinal ganglion cells in the goldfish. As shown in Fig. 8, we found responses similar to those recorded by de Valois. In fact, it

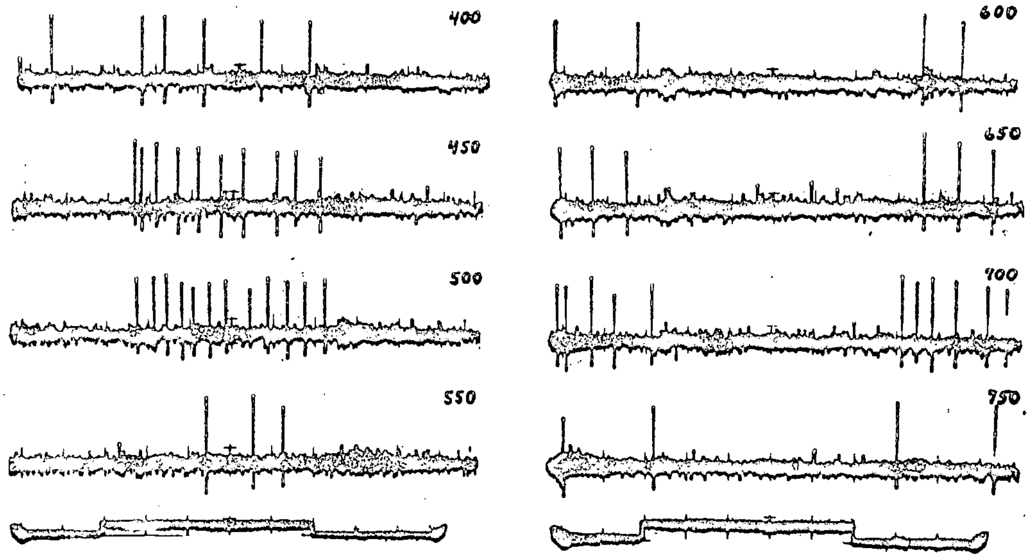

FIG. 8. Responses of a single ganglion cell of the goldfish at various wavelengths (shown at the right of each trace in mμ). Spikes occurring before the onset of a stimulus are off-responses from a previous stimulus. A repetitive 0·5 sec flash was applied at 2-sec intervals to keep the retina light adapted. The records shown are taken from a series of several hundred nearly identical responses. (From WAGNER *et al.*, 1960.)

was possible to obtain a shift in some units from a pure "on"- to a pure "off"-response with a wavelength change of less than 10 mμ, as shown in Fig. 9. This appears to indicate the existence of a very precise wavelength discriminating mechanism.

Figure 10 is a threshold vs. log intensity plot of a typical "green-on"–"red-off" ganglion cell. These curves bear little resemblance to the absorption spectra found by Marks. There is no reason why they should, since they represent the interaction of at least two populations of receptor cells at the ganglion cell level. DE VALOIS and JONES (1961) have indicated how the simple responses of the receptors can be greatly distorted by such interaction.

Units have been found that gave "on"-responses in the long wavelength region of the spectrum and "off"-responses in the short wavelength region of the spectrum. Others gave the opposite pattern of response. The "off"-response was associated with inhibition of activity during illumination regardless of whether it was spontaneous, the result of background illumination, or a continuation of the "on" burst elicited by the onset of a stimulus.

Thus we have tended to regard the "off"-response as a post-inhibitory rebound phenomenon which may serve to accentuate the termination of an inhibitory stimulus. Whatever its importance, it is clear that, in the goldfish at least, the information with regard to wavelength is carried up the optic nerve in the form of discharges of the axons of a population of ganglion cells which are acted upon by groups of receptors having sensitivities in different parts of the spectrum. These groups of receptors, presumably acting through the bipolar

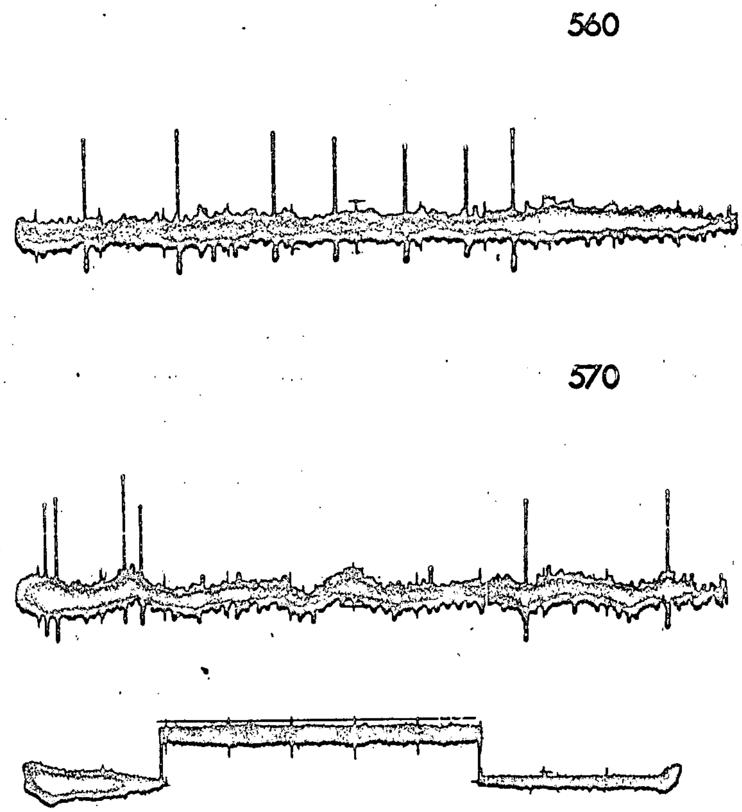

FIG. 9. Change from pure "on"-response to pure "off"-response with a wavelength shift of only 10 mμ. Same ganglion cell as that whose responses are shown in Fig. 8. (From WAGNER *et al.*, 1960.)

cells, exert either excitatory or inhibitory effects on the ganglion cells. For example, a given ganglion cell may be excited mainly by a group of red-sensitive receptors and inhibited mainly by a group of green-sensitive receptors. Other cells may be oppositely affected.

Some ganglion cells show no wavelength discriminatory responses at all: they have about equal on- and off-thresholds throughout the spectrum. It would be expected that such cells receive equal amounts of both excitatory and inhibitory connections from each type of receptor. A few cells respond by a maintained discharge during steady illumination. These presumably have only excitatory connections. Others discharge in the dark and responses are inhibited throughout prolonged illumination. These presumably have only inhibitory synapses.

Thus we have at the level of the *S*-potential, and later on at the level of the optic nerve fiber discharge, elements that behave in a way consistent with Hering's hypothetical red–green, blue–yellow, and black–white processes. Yet these elements are found in retinae of animals which have been shown to have three kinds of cones maximally sensitive in three spectral regions in accordance with Young's original predictions. Thus, as the originators of the various stage or zone theories have predicted, a retina may be consistent with the Young theory of color vision at the receptor cell level and with the Hering theory at the level of the optic nerve fibers (JUDD, 1951; SVAETICHIN and MACNICHOL, 1958; BRINDLEY, 1960; MARRIOTT, 1962.)

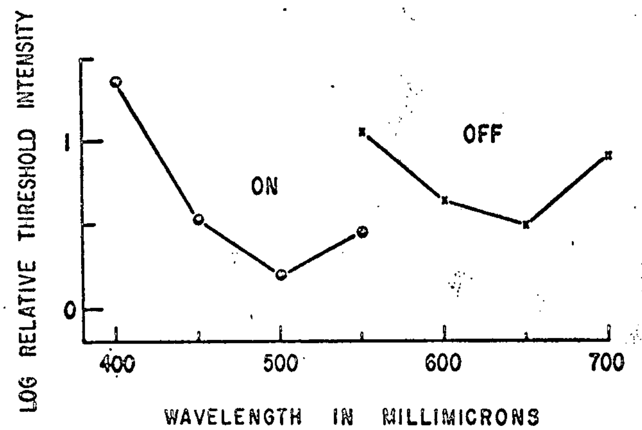

FIG. 10. Thresholds of on- and off-responses of one impulse as a function of the logarithm of the energy of an 0·5 sec stimulus at various wavelengths. Log intensity of zero represents 0·2 μ W/cm^2. (From WOLBARSHT *et al.*, 1961.)

DISCUSSION

At present there is some question as to whether the same mechanisms operate in the primates, since we have not yet recorded either bleaching or action spectra from single cones in any of these animals. However, the three cone pigments found by RUSHTON (1961), the three receptor processes uncovered by the increment threshold method of STILES (1959) and by the artificial monochromacy method of BRINDLEY (1960) furnish a strong presumption that similarities will be found. Furthermore, DE VALOIS *et al.* (1962), by partial bleaching experiments, have derived response functions from the lateral geniculate cells of the monkey that probably represent rather closely the action spectra of the chlorolabe and erythrolabe pigments in these animals. In addition, HUBEL and WIESEL (1960) have shown by recording from microelectrodes inserted into the monkey optic nerve that the pattern of responses recorded by de Valois in the lateral geniculate body is already encoded in the same form in the retina. Thus, the retinal mechanisms in the fish, monkey and man are not likely to differ very greatly.

It may be that Granit's modulators, which are responses to very narrow regions of the spectrum recorded by him in the retinae of some animals, represent a later step in the processing of retinal data than the wavelength sensitive "on–off" responses. Similar responses were recorded by DE VALOIS *et al.* (1958) from certain layers of the monkey's lateral geniculate body. They have not been recorded from the retinal ganglion cells of either monkeys or fishes.

In summary, it appears that the broad outlines of the retinal mechanism of color vision have now been determined by conceptually simple and direct, though sometimes difficult, experiments. The results conform to some rather ancient predictions which were supported, though not conclusively proven, by a wealth of psychophysical data.

For the sake of brevity many important details have been left out of this paper. Many more experiments need to be done before our knowledge is complete, but current progress gives promise that we will soon have a thorough knowledge of the mechanism responsible for that most interesting and useful phenomenon, color vision.

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